

SOME SPECIES OF *PYTHIUM* ISOLATED FROM CULTIVATED SOILS IN NORTHERN FRANCE

Bernard PAUL

Laboratoire de Mycologie, UFR Science, Université de Bourgogne,
B.P. 138, 21004 Dijon, France.

ABSTRACT - Nine species of *Pythium* including *P. echinulatum*, *P. irregulare*, *P. mamillatum*, *P. minor*, *P. ostracodes*, *P. torulosum*, *P. oligandrum*, *P. ultimum*, and *P. rostratum* isolated from cultivated soils in northern France are described. Some of these are new to this country. Taxonomic and morphological details of the 9 species of fungi are discussed.

RÉSUMÉ - Neuf espèces de *Pythium* notamment *P. echinulatum*, *P. irregulare*, *P. mamillatum*, *P. minor*, *P. ostracodes*, *P. torulosum*, *P. oligandrum*, *P. ultimum*, et *P. rostratum* ont été isolées à partir de sols cultivés dans le nord de la France. Quelques unes d'entre elles s'avèrent être nouvelles pour ce pays. Les détails morphologiques et taxonomiques de ces neuf espèces font l'objet de ce présent article.

KEY WORD - *Pythium*, sporangia, oogonia, antheridia, oospore.

INTRODUCTION

A perusal of the literature on the genus *Pythium* shows that very little work has been done on this important genus in France. Although works on the ecological and pathological aspect of *Pythium* have been carried out (Roze & Cornu, 1869; Moreau & Moreau, 1958; Bouhot, 1975; Montfort & Rouxel, 1988), have a description of *Pythium violae* as the causal organism for the «cavity spot disease» of carrots, and that of Forbes & Davet (1990) on the pathogenicity of *Pythium ultimum*, *P. sylvaticum*, and *P. irregulare* on soyabean roots, but none of these have a taxonomic treatment of the said species. An attempt is underway towards the taxonomy of the genus *Pythium* in France. A project on the isolation, identification, and preservation of pythiaceous fungi has been undertaken at the university of Bourgogne in Dijon. A number of soil samples from the region of Lille and Compiègne were examined and in this report nine species of *Pythium* are treated. All the cultures of the described fungi are being maintained at the Laboratoire de Mycologie, Université de Bourgogne, Dijon, France.

MATERIALS AND METHODS

Soil samples were collected in sterilized capped bottles and brought to the laboratory. Fungi were isolated by baiting with boiled hemp-seed halves introduced to a

soil suspension in water (Paul, 1986, 1987). Temperature/growth relations were observed on potato carrot agar (PCA) and corn meal agar (CMA). Benomyl (5 mg/l) was used to suppress the growth of *Fusarium* like fungi (Paul, 1991). Identification was done with the help of keys and descriptions of Middleton (1943), Waterhouse (1967), Plaats-Niterink (1981) and Dick (1990).

RESULTS AND OBSERVATIONS

Pythium torulosum Coker & Patterson (Figs. 1-5, & Fig. 59).

Colonies on CMA and PCA submerged, on PCA showing a rosette pattern and growing with an average daily growth rate of 15 mm at 25°C. Main hyphae upto 5-6 µm wide. **Sporangia** consisting of filamentous inflated, toruloid elements, vesicles and zoospores readily formed between 15-20°C, encysted zoospores measure about 7 µm in diam. **Oogonia** terminal or at times intercalary, globose or sub-globose, 12-25 (\bar{x} x 19 ± 0.6) µm diam. **Antheridia** mostly monoclinal, 1-2 per oogonium, antheridial cells making apical contact with the oogonia. **Oospores** globose, plerotic, single, 10-24 (\bar{x} .16 ± 0.6) µm diam. Wall 1-2.5 µm in thickness.

Pythium torulosum was isolated only twice from the region of Lille. The above description is that of F-80. Apart from slightly bigger oogonia and oospores, all the other characters of this isolates resembles with the description of *P. torulosum* found elsewhere (Plaats-Niterink, 1981). This is the first taxonomic treatment of the species in France.

Pythium rostratum Butler (Figs 6-9)

Colonies on CMA and PCA submerged, showing a chrysanthemum pattern on PCA. It is a slow growing fungus with an average daily rate of 8.5 mm at 25°C on this medium. Main hyphae upto 7-8 µm wide. **Sporangia** globose, ovoid, limoniform to cylindrical, terminal, intercalary, or catenulate, 18-32 µm diam. (\bar{x} 26.3 ± 0.6). **Oogonia** smooth walled, mostly intercalary or in chains, globose, ovoid, limoniform, ellipsoidal, 17-32 (\bar{x} 23.9 ± 0.6) µm diam. **Antheridia** mostly hypogynous, monoclinal and sessile, 1-2 per oogonium. **Oospores** globose, rarely oval, plerotic and aplerotic, usually single, rarely two per oogonium, 16-25 (\bar{x} 19.6 ± 0.6) µm diam. Wall 1.5-3 µm thick.

Pythium rostratum was originally isolated from garden soil in France (Butler, 1907). It is a very common species inhabiting the soil and was isolated from soil samples taken in the Compiègne as well as the Lille areas. Most of the morphological characters of the above described isolate (F-83) resemble to those found in the literature. The only difference worth mentioning is the presence of longer somewhat rectangular sporangia which can attain a length of up to 40 µm instead of 27 µm recorded by Plaats-Niterink (1981).

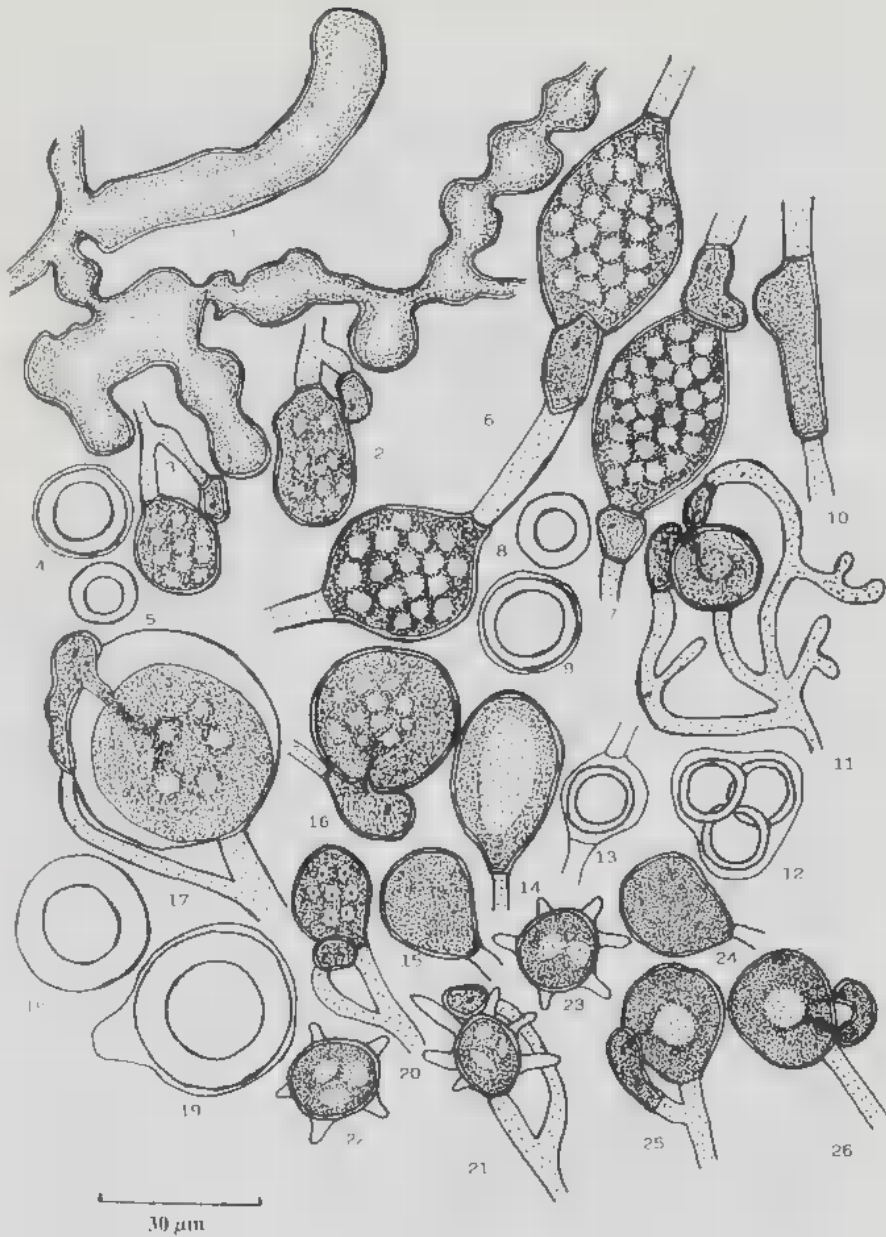
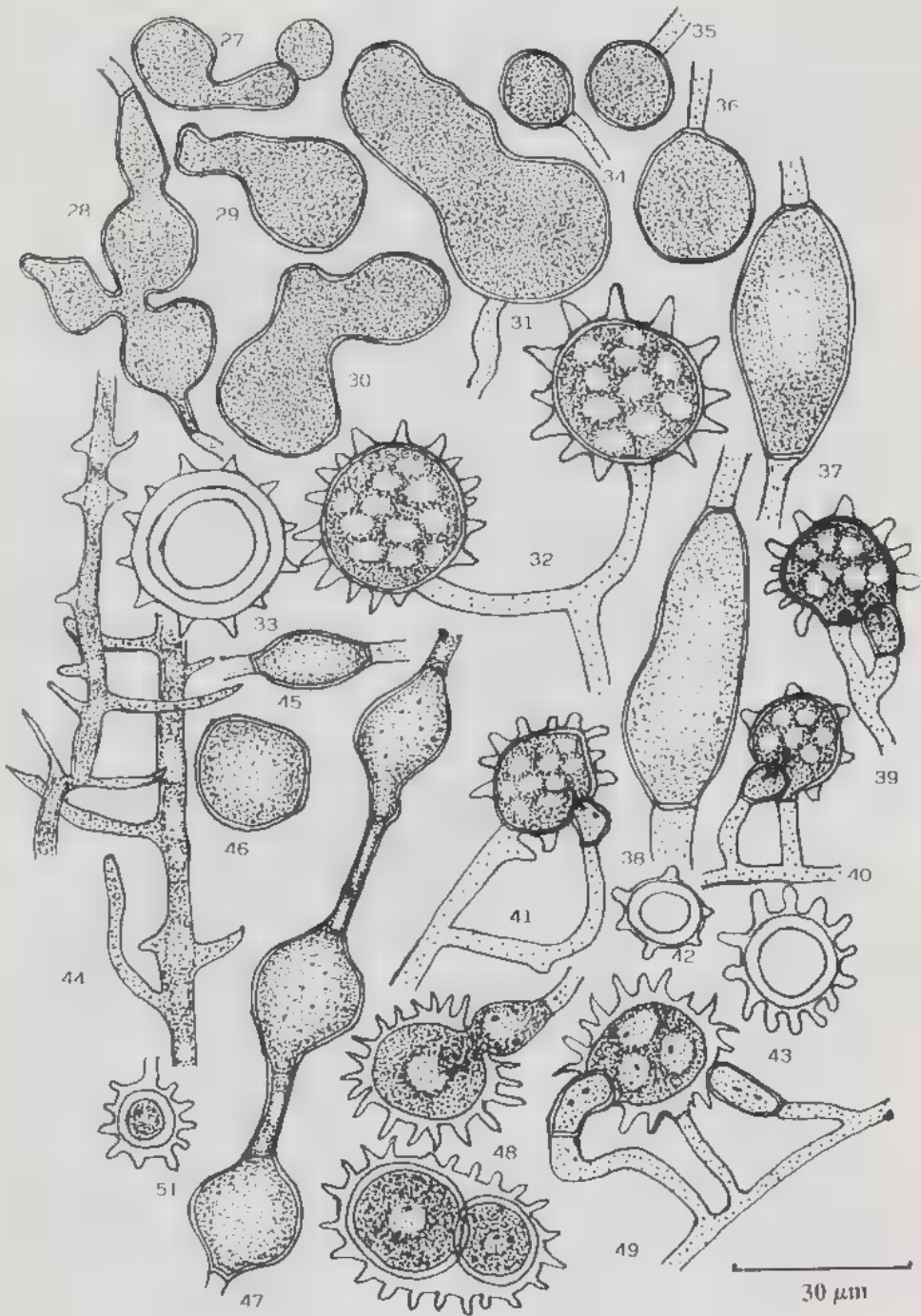


Figure 1-5: *Pythium torulosum*. 1: sporangia, 2-3: oogonia with monoclinal antheridia, 4-5: oospores; Figs 6-9: *Pythium rostratum*. 6: intercalary oogonia, 7: oogonium with hypogynous antheridia, 8-9: oospores; Figs 10-13: *Pythium minor*. 10: hyphal body, 11: oogonia with branched antheridia, 12-13: oospores; Figs 14-19: *Pythium ostracodes*. 14-15: sporangia, 16-17: oogonia, 18-19: oospores; Figs 20-23: *Pythium irregulare*. 20: smooth walled oogonia, 21: spiny oogonia with antheridia, 22-23: spiny oogonia; Figs 24-26: *Pythium ultimium*. 24: hyphal body, 25-26: oogonia with antheridia.



***Pythium minor* Ali-Shatayeh & Dick** (Figs 10-13, & Figs 52-53).

Colonies on CMA and PCA submerged, showing an indistinct rosette pattern on PCA. Daily growth rate on PCA at 25°C is 11 mm. Main hyphae upto 6 µm wide. **Sporangia** and zoospores not formed. **Hyphal bodies** produced abundantly on solid as well as in water on hemp seed halves. These are usually intercalary, catenulate, rarely terminal, globose, ovoid to cylindrical, the elongated ones are at times hardly thicker than the vegetative hyphae but filled with denser protoplasm, measuring 9-20 (\bar{x} 12.4 ± 0.6) µm diam. **Oogonia** terminal, infrequently intercalary, globose, ovoid, smooth walled, measuring 14-25 (\bar{x} 15.9 ± 0.6) µm diam. **Antheridia** monoclinal, much branched, giving a coralloid appearance and growing towards the oogonium, providing 1-2 antheridial cells to the latter. **Oospores** globose, 1-3 per oogonium, plerotic, rarely aplerotic, 10-15 (\bar{x} 12.7 ± 0.6) µm diam. with a very thin wall of 0.5-1 µm. Description isolate no. F-10.

Pythium minor is a very common species in the north of France. It was isolated twice in Compiègne and 5 times from soil samples taken at different places in the region of Lille. After its discovery in England (Ali-Shatayeh & Dick, 1985) it has not been reported from elsewhere. Branched antheridia forming a coralloid structure around the oogonia, small oogonia and oospores, and slow growth makes it easily distinguishable from other species. However there are some differences between this isolate and the one described by Ali-Shatayeh & Dick (1985): the hyphal bodies in this case are much smaller (9-20 µm instead of 20-40 µm). More than 3 oospores per oogonium were not observed in the isolates from France as compared with 6 from that described from England. This is the first report of its occurrence in France.

***Pythium ostracodes* Drechsler** (Figs 14-19, & 54-55).

Colonies on CMA and PCA producing abundant aerial mycelium. Daily growth rate on PCA at 25°C 8-9 mm. Main hyphae upto 7 µm wide. **Sporangia** spherical, ovoid, at times with an apical papilla 13-33 µm diam. (\bar{x} 23 ± 0.6). **Oogonia** smooth walled, globose, terminal or intercalary, 15-40 (\bar{x} 26 ± 0.6) µm diam. **Antheridia** 1-2 per oogonium, monoclinal with long antheridial cells that apply to the oogonium to most of its surface, antheridial cells upto 25 µm long and 6 µm wide. **Oospores** globose, plerotic, one per oogonium, 14-39 (\bar{x} 24 ± 0.6) µm diam., and provided with a very thick wall of 4-7 µm.

Pythium ostracodes isolated only once, from a soil sample taken from a wheat field on Lille (No F-66). It was first described from wheat in Texas (Drechsler, 1943) and later on it was isolated from rhizomes of latus in Japan (Takahashi *et al.*, 1965). This is the first report of its presence in France. This species can be separated from

Fig. 27-33: *Pythium oligandrum*. 27-31: irregular elements of the contiguous sporangia. 32: terminal oogonia, 33: aplerotic oospores; Figs 34-43: *Pythium mamillatum*. 34-38: sporangia. 39-41: ornamented oogonia with antheridia, 42-43: oospores. Figs 44-51: *Pythium echinulatum*. 44: vegetative hyphae, 45-47: sporangia, 48: oogonia with hypogynous antheridia, 49: oogonia with monoclinal antheridia, 50: oogonia containing two oospores, 51: oogonia with single oospore.

other species by its slow growth, large plerotic oospores, and long, laterally applied antheridia. This isolate has all these characters, but it does not sporulate. Zoospores were not observed, moreover its average oogonial and oosporal sizes are smaller (26 & 24 μm instead of 35 & 32.5 μm , respectively), and oospore wall thicker (upto 7 μm instead of 5 μm) than those described by Plaats-Niterink (1981).

***Pythium irregulare* Buisman** (Figs 20-23).

Colonies on CMA & PCA producing profuse aerial mycelium. Daily growth rate on PCA at 25°C is 24.5 mm. Main hyphae up to 5-6 μm wide. **Hyphal swellings** globose to ovoid, sometimes provided with single papilla, terminal or intercalary. 14-26 μm diam. (\bar{x} 19 \pm 0.6). Sporangia and zoospores were not formed. **Oogonia** terminal or intercalary, globose to somewhat elongated, 13-21 (\bar{x} 20 \pm 0.6) μm diam., smooth walled or provided with a varying number of blunt conical projections up to 12 μm long, 0-6 per oogonium. **Antheridia** mostly monoclinal, 1-3 per oogonium. **Oospores** globose, plerotic or aplerotic, 12-20 (\bar{x} 16 \pm 0.6) diam. Wall 1.5-2 μm thick.

Pythium irregulare was isolated only on three locations, once in Compiègne and twice in the region of Lille. Because of the presence of both spiny and smooth-walled oogonia, it is an easily recognisable fungus. However, in water cultures, the number of ornamented oogonia was larger than that of the smooth walled ones. The features of the three isolates agree well with the description of this species reported in literature (Plaats-Niterink, 1981). The characteristics given above are those of culture no. F-75 isolated from ■ soil sample taken in the region of Lille.

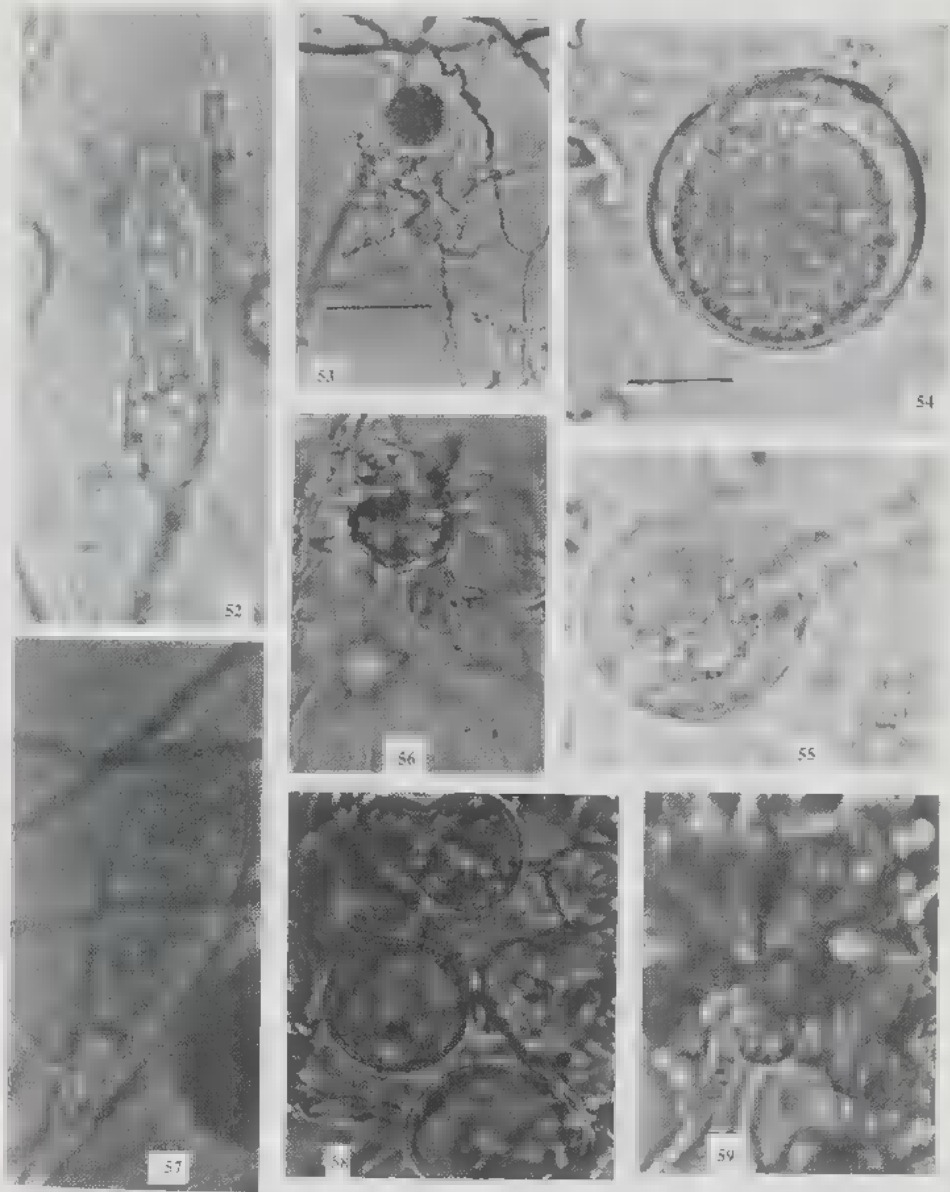
***Pythium ultimum* Trow** (Figs 24-26).

Colonies on CMA forming profuse aerial mycelium and on PCA showing an indistinct radiate pattern. Average daily growth rate on PCA at 25°C is 28 mm. Main hyphae up to 10 μm wide. **Sporangia** and zoospores not formed. Hyphal swellings formed plentifully and are globose, intercalary, sometimes terminal 18-25 μm diam. (\bar{x} 20 \pm 0.6). **Oogonia** terminal or intercalary, spherical, smooth walled, 19-31 (\bar{x} 23.4 \pm 0.6) μm diam. **Antheridia** mostly monoclinal originating immediately below the oogonium, sessile, saclike, infrequently hypogynous, rarely declinal, 1-2 per oogonium. **Oospores** spherical, aplerotic, at times plerotic, single, 17-24 (\bar{x} 20.6 \pm 0.6) μm diam. Wall 1-3 μm thick.

Pythium ultimum is an aggressive plant parasite and was isolated many times in Compiègne as well as Lille. The above description is that of isolate F-39.1 isolated from Lille. In spite of some variations of oogonium and oospore dimensions, and the presence of some plerotic oospores together with the usual aplerotic ones, all the other characters of this fungus fit closely the description of *P. ultimum* found in the literature (Plaats-Niterink, 1981).

***Pythium oligandrum* Drechsler** (Figs 27-33 & Fig. 58)

Colonies on CMA forming some aerial mycelium, submerged on PCA showing an indistinct radiate pattern. Average daily growth rate on PCA at 25°C is 25 mm. Main hyphae upto 8 μm wide. **Sporangia** intercalary, composed of spherical to irregular, contiguous elements of up to 35 μm wide and 60 μm long. Zoospores not formed. **Oogonia** mostly terminal, spherical, ornamented with conical, acutely tipped spines.



Figs 52-53: *Pythium minor*. 52: elongated hyphal body, 53: oogonia with branched antheridia; Figs 54-55: *Pythium astracodes*. 54: oogonia with antheridia, 55: plerotic oospore; Figs 56-57: *Pythium echinulatum*. 56: oogonia with hypogynous antheridia, 57: elongated intercalary sporangia; Fig. 58: *Pythium oligandrum*, contiguous sporangia, Fig. 59: *Pythium torulosum*: inflated elements of sporangial complex. (Fig. 53, bar = 40µm, all other figures, bar = 16 µm).

20-31 (\bar{x} 24.4 \pm 0.6) μm diam., with spines of upto 6 μm long and upto 3.5 μm in basal diameter. **Antheridia** absent. **Oospores** spherical, aplerotic, one per oogonium, 17-27 (\bar{x} 22.6 \pm 0.6) μm diam. with a wall approximately 2 μm thick.

Pythium oligandrum is abundant in the North of France. It was isolated from soil samples in the Compiègne as well as Lille regions. The above description is that of isolate F-81 isolated from Lille. There are some minor differences in the oogonial and oosporal dimensions of this isolate and those found in the literature, however all the other morphological characters of this fungus are identical. This is the first taxonomic treatment of *Pythium oligandrum* isolated in France.

Pythium mamillatum Meurs (Figs 34-43).

Colonies on CMA & PCA produces some aerial mycelium and shows a rosette pattern on the latter. Average daily growth on PCA at 25°C is 20 mm. Main hyphae are up to 7 μm wide. **Sporangia** or hyphal swellings are globose, ovoidal, ellipsoidal to somewhat cylindrical, intercalary, at times terminal, measuring 13 to 28 μm diam. (\bar{x} 19.4 \pm 0.6). **Oogonia** intercalary or terminal, globose to slightly ovoidal, provided with blunt to conical, and at times, curved spines 2-6 μm long and 1-3 μm broad. Oogonia 13-24 μm in diam. (\bar{x} 18.3 \pm 0.6). **Antheridia** mostly monoclinal rarely dichlinal, usually one, infrequently two per oogonium, antheridial cells clavate making apical contact with the female gametangia. **Oospores** globose, plerotic, one per oogonium, measuring 12-22 (\bar{x} 16.4 \pm 0.6) μm diam., provided with a moderately thin wall of up to 2 μm .

The above description is of isolate no F-60, isolated from the region of Compiègne. The other two isolates of this species were collected in Lille. All the features of this fungus resembles to those found in the literature. The only difference is the absence of zoospores inspite of the presence of sporangia. The fungus failed to sporulate despite of repeated flooding in cultures with distilled water, soil extract water and maintenance at different temperatures. This is the first taxonomic description of *Pythium mamillatum* isolated in France.

Pythium echinulatum Matthews (Figs 44-51 & Figs 56-57)

Colonies on CMA & PCA submerged showing an indistinct radiate pattern. Daily growth rate on PCA at 25°C 10 mm. Main hyphae upto 7-8 μm wide. **Sporangia** globose to cylindrical, terminal or intercalary, more often in chains, 11-25 μm diam. (\bar{x} 17 \pm 0.6). **Oogonia** terminal, intercalary, catenulate, globose to cylindrical, provided with acute conical spines 3-9 μm long. Oogonia 9-26 (\bar{x} 18 \pm 0.6) μm diam. (excluding spines). **Antheridia** mostly hypogynous at times mono and dichlinal, 1-2 per oogonium. **Oospores** globose, rarely oval, plerotic or aplerotic, usually single, rarely two per oogonium, 6-24 (\bar{x} 15 \pm 0.6) μm diam. Wall 1.5-2 μm thick.

Pythium echinulatum was frequently isolated from the north of France. Nevertheless this species shows a great morphological variation. Acute conical spines on the oogonia, hypogynous antheridia, aplerotic and plerotic oospores were common in all the isolates, but the presence of catenulate sporangia and oogonia were not constantly found in all the isolates.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. A.J. Van Der Plaats Niterink who has been kind enough to review this article.

REFERENCES

- ALI-SHTAYEH M.S. and DICK M.W., 1985 - Five new species of *Pythium* (Perenosporomycetidae). *Bot. J. Linn. Soc.* 91: 297-317.
- BOUHOT D., 1975 - Recherches sur l'écologie des champignons parasites dans le sol. 5. Une technique sélective d'estimation du potentiel infectieux des sols, terreaux et substrats infestés par *Pythium* sp., études qualitatives. *Ann. Phytopathol.* 7: 9-18.
- BUTLER E.J., 1907 - An account of the genus *Pythium* and some Chytridiaceae. *Mem. Dept. Agric., India, Bot. ser.* 1 (5): 1-162.
- DICK M.W., 1990 - Keys to *Pythium*.. University of Reading Press, Reading. 64 p.
- FORBES G.A. & DAVET P., 1990 - Characterization and pathogenicity on seedlings of *Pythium* species isolated from soybean roots in the Toulouse area. *Agronomie* 10: 825-830.
- DRECHSLER C., 1943 - Two species of *Pythium* occurring in southern states. *Phytopathology* 33: 261-299.
- MIDDLETON J.T., 1943 - The taxonomy, host range, and geographical distribution of the genus *Pythium*. *Mem. Torrey Bot. Club* 20: 1-171.
- MONTFORT F. & ROUXEL F., 1988 - La maladie de la «tache» de la carotte due à *Pythium violae* Chesters et Hickman: données symptomatologiques et étiologiques. *Agronomie* 8 (8): 701-706.
- MOREAU C. & MOREAU M., 1958 - Le «blast» des jeunes palmiers à huile. Observations sur le système racinaire de l'hôte et sur ses parasites. *Rev. Mycol.* 23: 201-232.
- PAUL B., 1986 - An aquatic species, *Pythium taruloides* sp. nov., from Algeria. *Trans. Brit. Mycol. Soc.* 89 (2): 195-198.
- PAUL B., 1987 - A new species of *Pythium* with ornamented oogonia from Algeria. *Mycologia* 79 (5): 797-802.
- PAUL B., 1991 - *Pythium folliculosum*: A new species from the bank of Lake Zürich. *Mycol. Helv.* 4: 203-208.
- PLAATS-NITERINK A.J. van der, 1981 - Monograph of the genus *Pythium*. *Stud. Mycol. Baarn* 21: 1-242.
- ROZE E. & CORNU M., 1869 - Sur deux nouveaux types génériques pour les familles des Saprolegniées et Péronosporées. *Ann. Sci. Nat., Bot.*; Série 5: (11): 2-91.
- TAKAHASHI M., OHUCHI A. & ALICBUSAN R.V., 1965 - Ecologic and taxonomic studies on *Pythium* as pathogenic soil fungi. 6. Some species of *Pythium* causing rhizome rot of hindu lotus. *Ann. Phytopathol. Soc. Japan* 30: 186-191.
- WATERHOUSE G.M., 1967 - Key to *Pythium* Pringsheim. *Mycol. pap.* 109: 1-15.